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Mercedes F. dipasupil (Signature)

U.S. PATENT APPLICATION

on

NON-STEROIDAL FARNESOID X RECEPTOR MODULATORS AND METHODS FOR THE USE THEREOF

by

Michael R. Downes,

Ronald M. Evans,

Robert Hughes,

K. C. Nicolaou, and

Anthony J. Roecker

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Attorneys

Foley & Lardner

P.O. Box 80278

San Diego, CA 92130-0278

NON-STEROIDAL FARNESOID X RECEPTOR MODULATORS AND METHODS FOR THE USE THEREOF

FIELD OF THE INVENTION

[0001] The present invention relates to new chemical entities. In a particular aspect, the present invention relates to non-steroidal modulators of farnesoid X receptors (FXR). In another aspect, the present invention relates to methods for modulating FXR-mediated processes employing the novel compounds described herein.

BACKGROUND OF THE INVENTION

[0002] The efficient regulation of cholesterol biosynthesis, metabolism, acquisition and transport is an essential function of mammalian cells. High levels of cholesterol are associated with atherosclerosis, a leading cause of death in the western world and a major risk factor correlated with the occurrence of coronary heart disease and stroke. Until recently, recommendations for the treatment of hypercholestemia were focused on the use of statins, which inhibit the de novo biosynthesis of cholesterol, and the use of bile acid sequestering agents. While statin-based agents are still in widespread use as cholesterol-lowering drugs, an evolving understanding of the mechanisms controlling cholesterol homeostasis has led to new molecular targets as candidates in therapeutic intervention.

[0003] Cholesterol metabolism is controlled through a complex feedback loop involving cholesterol itself and bile acids (which are primary oxidation products), and through secretion in the gut, the single most critical regulators of cholesterol absorption. The nuclear receptors LXR (liver X receptor) and FXR (farnesoid X receptor) are the specialized sensors of cholesterol and bile acids that control transcription of networks encoding key metabolic enzymes. For example activation of LXR by oxysterols (i.e., mono-oxygenated cholesterol metabolites) leads to the up-regulation of CYP7A1, the enzyme that catalyzes the rate limiting step in the conversion of cholesterol to bile acids. In turn, bile acids such as chenodeoxycholic acid (CDCA, **1**, Figure 1) are potent ligands for FXR, whose activation leads to down-regulation of CYP7A1, leading to

the completion of the feedback circuit. In this circuit FXR induces the expression of a transcriptional repressor SHP (small heterodimer partner) which in turn binds to LRH-1 (liver receptor homolog), which is required in CYP7A activation. Additionally, both LXR and FXR are implicated in the regulation of several other gene products involved in cholesterol absorption, metabolism and transport.

[0004] Thus, the identification of potent, selective, small molecule FXR agonists, partial agonists and antagonists would be powerful tools and would have many potential applications. For example, such compounds would facilitate the *in vivo* analysis of FXR physiology *in vivo*. In addition, such compounds, in conjunction with DNA arraying technology, might allow for the discovery of new gene products under the control of FXR. Further, FXR modulators might find potential utility in the treatment of cholestasis and other disease states associated with aberrant levels, flow and release of bile acids. Moreover, in the absence of a crystal structure of FXR, a thorough structure-activity relationship (SAR) study of ligands that modulate the activity of FXR would allow for the delineation of the structural requirements for ligand binding and might aid in the design of future ligands and potential therapeutics.

SUMMARY OF THE INVENTION

[0005] In accordance with the present invention, the identification of novel potent FXR activators is described. Initial screening of a 10,000-membered, diversity-orientated library of benzopyran containing small molecules for FXR activation utilizing a cell-based reporter assay led to the identification of several lead compounds owning low micromolar activity (EC_{50}'s = 5 – 10 μM). These compounds were systematically modified employing parallel solution-phase synthesis and solid-phase synthesis to provide numerous compounds that potently activate FXR. Two derivatives of invention compounds, bearing stilbene or biaryl moieties, contain members that are the most potent FXR agonists reported to date in cell-based assays. These compounds are useful as chemical tools to further define the physiological role of FXR as well as therapeutic leads for the treatment of diseases linked to cholesterol, bile acids and their metabolism and homeostasis.

BRIEF DESCRIPTION OF THE FIGURES

[0006] Figure 1 presents the structures of several natural and synthetic agonists of FXR.

[0007] Figures 2A and 2B present the structures of selected compounds identified as potential FXR agonists. The boxed compounds represent the most potent RXR agonists from among those tested in the original 10,000 membered benzopyran-based natural product-like library.

[0008] Figure 3 collectively illustrates the solid-phase synthesis of a focused library of benzopyran containing small molecules as potential FXR agonists. Figure 3A illustrates the synthetic scheme used for library construction; Figure 3B illustrates the scaffolds used; Figure 3C illustrates the electrophiles used in this synthesis; and Figure 3D illustrates the amines used in this synthesis.

[0009] Figure 4 identifies selected regions of a model benzopyran compound for SAR evaluation.

[0010] Figure 5 summarizes the examination of SAR for variations in Region I of the model benzopyran compound illustrated in Figure 4.

[0011] Figure 6 illustrates a representative procedure for the preparation of the Region I modified compounds referred to in Figure 5.

[0012] Figure 7 illustrates a solution phase synthetic procedure for the preparation of the Region I modified compounds referred to in Figure 5.

[0013] Figure 8 illustrates an alternate solution phase synthetic procedure for the preparation of the Region I modified compounds referred to in Figure 5.

[0014] Figure 9 illustrates another alternate solution phase synthetic procedure for the preparation of the Region I modified compounds referred to in Figure 5.

[0015] Figure 10 summarizes the examination of SAR for variations in Region II of the model benzopyran compound illustrated in Figure 4.

[0016] Figure 11 illustrates a solution phase synthetic procedure for the preparation of the Region II modified compounds referred to in Figure 10.

[0017] Figure 12 summarizes the examination of SAR for variations in Region III of the model benzopyran compound illustrated in Figure 4.

[0018] Figure 13 illustrates a solution phase synthetic procedure for the preparation of the Region III modified compounds referred to in Figure 12.

[0019] Figure 14 illustrates an alternate solution phase synthetic procedure for the preparation of the Region III modified compounds referred to in Figure 12.

[0020] Figure 15 illustrates a synthetic protocol for the preparation of invention compound **102**.

[0021] Figure 16 summarizes the examination of SAR for additional variations in Region III of the model benzopyran compound illustrated in Figure 4.

[0022] Figure 17 illustrates a solution phase synthetic procedure for the preparation of the Region III modified compounds referred to in Figure 16.

[0023] Figure 18 illustrates an alternate solution phase synthetic procedure for the preparation of the Region III modified compounds referred to in Figure 16.

[0024] Figure 19 summarizes the activities of various invention compounds in a functional assay.

[0025] Figure 20 illustrates a synthetic protocol for the preparation of invention compound **105**.

[0026] Figure 21 illustrates yet another solution phase synthetic procedure for the preparation of the Region III modified compounds referred to in Figure 16.

[0027] Figure 22 illustrates a synthetic protocol for the preparation of cinnamate derivatives according to the invention.

[0028] Figure 23 illustrates a synthetic protocol for the preparation of acyl group analogs of invention cinnamate derivatives.

[0029] Figure 24 illustrates a synthetic protocol for the preparation of Region III variants of cinnamate derivatives according to the invention.

[0030] Figure 25 illustrates a synthetic protocol for the preparation of Region III ring analogs of cinnamate derivatives according to the invention.

[0031] Figure 26 illustrates a solid phase synthetic procedure for the preparation of biaryl and stilbene cinnamate derivatives according to the invention.

[0032] Figure 27 presents the structures of styrenes and boronic acids used in the construction of the library contemplated by Figure 26.

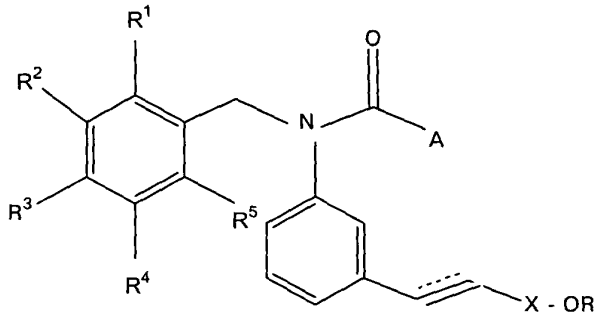
[0033] Figure 28 summarizes the activities of numerous stilbene and biaryl derivatives of invention compounds.

[0034] Figure 29 summarizes the structural requirements for potent FXR activation.

[0035] Figure 30 summarizes the efficacy of the functional assay for the identification of FXR agonists, using the known FXR agonist, chenodeoxycholic acid (CDCA).

DETAILED DESCRIPTION OF THE INVENTION

[0036] In accordance with the present invention, there are provided compounds having the structure:



wherein:

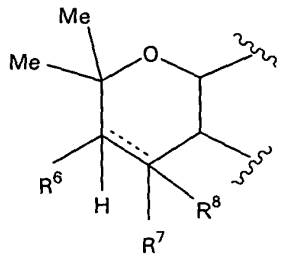
A is a C3 up to C8 branched chain alkyl or substituted alkyl group, a C3 up to C7 cycloalkyl or substituted cycloalkyl, an optionally substituted aryl or an optionally substituted heteroaryl,

X is $-C(O)-$ or $-CH_2-$,

R is methyl or ethyl,

R^1 is H, hydroxy, alkoxy, benzoyloxy, mesityloxy, or $-OCH_2C(O)OC_2H_5$,

R^2 is H or R^2 can cooperate with R^3 to form a benzopyran, wherein the pyran ring has the structure:



wherein:

R^6 is not present if the pyran ring is unsaturated, or, if present, is selected from H, -OR, wherein R is alkyl or acyl, or R^6 can cooperate with R^7 to form a cyclic acetal, a cyclic ketal, or a cyclopropyl moiety, and

only one of R^7 and R^8 is present if the pyran ring is unsaturated, or R^7 and R^8 are independently H, carboxyl, cyano, hydroxy, alkoxy, thioalkyl, aryl, or R^7 and R^8 taken together comprise a carbonyl oxygen or an oxime nitrogen, or either R^7 or R^8 can cooperate with R^6 to form a cyclic acetal, a cyclic ketal, or a cyclopropyl moiety,

R^3 can cooperate with R^2 to form a benzopyran having the structure set forth above, or R^3 is alkenyl, optionally substituted aryl or heteroaryl, or optionally substituted arylalkenyl or heteroarylalkenyl,

R^4 is H or hydroxy, and

R^5 is H, hydroxy, alkoxy or aryloxy.

[0037] As employed herein, "alkyl" refers to saturated straight or branched chain hydrocarbon radical having in the range of 1 up to about 20 carbon atoms. "Lower alkyl" refers to alkyl groups having in the range of 1 up to about 5 carbon atoms. "Substituted alkyl" refers to alkyl groups further bearing one or more substituents selected from hydroxy, alkoxy (of a lower alkyl group), mercapto (of a lower alkyl group), cycloalkyl, substituted cycloalkyl, heterocyclic, substituted heterocyclic, aryl, substituted aryl, heteroaryl, substituted heteroaryl, aryloxy, substituted aryloxy, halogen, trifluoromethyl, cyano, nitro, nitrone, amino, amido, —C(O)H, acyl, oxyacyl, carboxyl, carbamate, dithiocarbamoyl, sulfonyl, sulfonamide, sulfuryl, and the like.

[0038] As employed herein, "alkenyl" refers to straight or branched chain hydrocarbyl groups having at least one carbon-carbon double bond, and having in the range of about 2 up to 20 carbon atoms, and "substituted alkenyl" refers to alkenyl groups further bearing one or more substituents as set forth above.

[0039] As employed herein, "alkoxy" refers to –O-alkyl groups having in the range of 2 up to 20 carbon atoms and "substituted alkoxy" refers to alkoxy groups further bearing one or more substituents as set forth above.

[0040] As employed herein, "cycloalkyl" refers to a cyclic ring-containing groups containing in the range of about 3 up to about 8 carbon atoms, and "substituted cycloalkyl" refers to cycloalkyl groups further bearing one or more substituents as set forth above.

[0041] As employed herein, "heterocyclic" refers to cyclic (i.e., ring-containing) groups containing one or more heteroatoms (e.g., N, O, S, or the like) as part of the ring structure, and having in the range of 3 up to 14 carbon atoms and "substituted heterocyclic" refers to heterocyclic groups further bearing one or more substituents as set forth above.

[0042] As employed herein, "aryl" refers to aromatic groups having in the range of 6 up to 14 carbon atoms and "substituted aryl" refers to aryl groups further bearing one or more substituents as set forth above.

[0043] As employed herein, "aryloxy" refers to –O-aryl groups having in the range of 6 up to 14 carbon atoms and "substituted aryloxy" refers to aryloxy groups further bearing one or more substituents as set forth above.

[0044] As employed herein, "arylalkenyl" refers to aryl-substituted alkenyl groups and "substituted arylalkenyl" refers to arylalkenyl groups further bearing one or more substituents as set forth above.

[0045] As employed herein, "heteroaryl" refers to aromatic groups having in the range of 4 up to about 13 carbon atoms, and at least one heteroatom selected from O, N, S, or the like; and "substituted heteroaryl" refers to heteroaryl groups further bearing one or more substituents as set forth above.

[0046] As employed herein, "heteroarylalkenyl" refers to heteroaryl-substituted alkenyl groups and "substituted heteroarylalkenyl" refers to heteroarylalkenyl groups further bearing one or more substituents as set forth above.

[0047] As employed herein, "acyl" refers to alkyl-carbonyl species.

[0048] As employed herein, "halogen" refers to fluoride, chloride, bromide or iodide atoms.

[0049] As employed herein, reference to "a carbamate group" embraces substituents of the structure $-O-C(O)-NR_2$, wherein each R is independently H, alkyl, substituted alkyl, aryl or substituted aryl as set forth above.

[0050] As employed herein, reference to "a dithiocarbamate group" embraces substituents of the structure $-S-C(S)-NR_2$, wherein each R is independently H, alkyl, substituted alkyl, aryl or substituted aryl as set forth above.

[0051] As employed herein, reference to "a sulfonamide group" embraces substituents of the structure $-S(O)_2-NH_2$.

[0052] As employed herein, "sulfuryl" refers to substituents of the structure $=S(O)_2$.

[0053] As employed herein, "amino" refers to the substituent $-NH_2$.

[0054] As employed herein, "monoalkylamino" refers to a substituent of the structure –NHR, wherein R is alkyl or substituted alkyl as set forth above.

[0055] As employed herein, "dialkylamino" refers to a substituent of the structure $-NR_2$, wherein each R is independently alkyl or substituted alkyl as set forth above.

[0056] As employed herein, reference to "an amide group" embraces substituents of the structure $-C(O)-NR_2$, wherein each R is independently H, alkyl, substituted alkyl, aryl or substituted aryl as set forth above. When each R is H, the substituent is also referred to as "carbamoyl" (i.e., a substituent having the structure $-C(O)-NH_2$). When only one of the R groups is H, the substituent is also referred to as "monoalkylcarbamoyl" (i.e., a substituent having the structure -C(O)-NHR, wherein R is alkyl or substituted alkyl as set forth above) or "arylcarbamoyl" (i.e., a substituent having the structure -C(O)-NH(aryl), wherein aryl is as defined above, including substituted aryl). When neither of the R groups are H, the substituent is also referred to as "di-alkylcarbamoyl" (i.e., a substituent having the structure $-C(O)-NR_2$, wherein each R is independently alkyl or substituted alkyl as set forth above).

[0057] In accordance with a particular embodiment of the present invention, presently preferred compounds are those wherein A is a C5-C7 cycloalkyl group.

[0058] In accordance with another particular embodiment of the present invention, presently preferred compounds are those wherein X is –C(O)-.

[0059] In accordance with yet another particular embodiment of the present invention, presently preferred compounds are those wherein R^1 is hydrogen.

[0060] In accordance with still another particular embodiment of the present invention, presently preferred compounds are those wherein R^2 and R^3 cooperate to form a benzopyran.

[0061] In accordance with a further particular embodiment of the present invention, presently preferred compounds are those wherein R^3 is alkenyl, thereby producing a cinnamate derivative.

[0062] In accordance with a still further embodiment of the present invention, presently preferred compounds are those wherein R^3 is an optionally substituted aryl or heteroaryl moiety, thereby producing biphenyl derivatives.

[0063] In accordance with yet another embodiment of the present invention, presently preferred compounds are those wherein R^3 is an optionally substituted arylalkenyl or heteroarylalkenyl moiety, thereby producing stilbene derivatives.

[0064] As there was, prior to the present invention, only one example of high affinity, non-steroidal agonist for FXR, GW4064 (**3**, Figure 1), the strategy adopted herein for identification of additional potent compounds involved screening a 10,000-membered library constructed around the privileged 2,2-dimethylbenzopyran scaffold. Such privileged structures are attractive starting points for lead compound discovery, particularly when there exists little structural information regarding the target, as they show good binding affinity toward a wide variety of enzymes and receptors. The initial hits discovered from screening of this library for FXR activation could be further modified for enhanced potency and pharmacological properties suitable for the applications mentioned above. Implementation of such a strategy is described herein, culminating in the discovery of numerous potent and selective activators of FXR.

[0065] Thus, in accordance with the present invention, a cell-based transcription assay was employed in which an FXR responsive promoter is linked to a luciferase reporter as the primary screen (see Example 1). In addition to ensuring that only cell permeable compounds were selected for further optimization, this approach allows for the detection of FXR activation in a natural system (i.e., correct folding of the protein and in the presence of a complete compliment of co-activators and co-repressors). Initial screening of a 10,000-membered combinatorial library of benzopyran-based small molecules in this high-throughput, cell-based assay for FXR activation produced several lead compounds whose structures are listed in Figure 2a (compounds **4 – 15**). Guided by the preliminary structure-activity relationships (SAR) gained from the evaluation of this initial library, a follow-up focused library of about 200 benzopyran-based compounds was designed and synthesized on solid support (see Figure 3). A selection of the most active compounds, possessing activities from 5 – 10 μM, discovered from this second

round of screening, is shown in Figure 2b (compounds **16** – **27**). Compounds **26** and **27** proved to be among the most active at this stage and were the subject of further modification as described below.

[0066] With initial lead compounds identified and validated, the stage was set for the systematic modification of the three regions of the lead structure shown in Figure 4. As detailed in the following sections, focused libraries were synthesized and screened in the cell-based assay in order to evaluate the structural requirements of each region of the molecule for potent FXR agonism. At this point parallel solution-phase chemistry was selected for the construction of additional focused libraries. This shift away from solid-phase chemistry provided maximum flexibility in efforts to rapidly and systematically modify each region of the lead molecules using smaller designed libraries.

Evaluation of Benzopyran Region I SAR

[0067] Most of the FXR agonists reported to date including CDCA (**1**), TTNPB (**2**) and GW4064 (**3**) (see Figure 1) contain a carboxylic acid moiety. It was reasoned, therefore, that incorporation of an acid unit within either region I, II or III of structure **26** (Figure 4) would confer increased potency upon this rather weak ligand (5 – 10 μM) identified via HTS. Guided by this reasoning, the SAR of region I was evaluated. Several compounds, displaying the acid unit in various positions, were synthesized (e.g., compounds **28**, **36**, **52**, **54** and **56**, Figure 5) and tested. None of these compounds, however, showed improved activation of FXR. Interestingly, compound **29**, bearing a meta methyl acrylate moiety, was a substantially better activator of FXR than compound **26**. The preparation of compound **29** is representative of the methods employed to construct these compounds and is described in Figure 6 (see Figures 7-9 for further experimental procedures). Thus, aldehyde **59** was selectively methylated (NaH, MeI), alkylated (2-methyl-3-butyn-2-ol, TFAA, DBU, $CuCl_2$), reduced (Lindlar, H_2) and thermally cyclized to yield benzopyran **60**. Reductive amination of aldehyde **60** with 3-bromoaniline ($NaCNBH_3$) followed by acylation with cyclopropanecarbonyl chloride (C_3H_5COCl, Et_3N) and palladium-mediated Heck coupling ($Pd_2(dba)3$, P(*o*-tol)$_3$, Et_3N) with methyl acrylate provided compound **29**.

[0068] In further refining the SAR of region I, it was observed that the location of the methyl acrylate moiety at the meta position was important for potent activation of FXR, as compound **53** (Figure 5) bearing a para methyl acrylate, does not activate FXR under the conditions tested. In order to further examine what functionality was tolerable at the meta position, the additional compounds shown in Figure 5 were synthesized. From biological screening of these compounds it became clear that the length and rigidity of the tether between the aromatic core and the interacting functionality (either methyl ester or methyl ether) are important for FXR agonism. For instance, compounds **41** and **45** appear to possess either too short or too long of a tether for potent activity; compounds **35** and **46 – 49** presumably cannot adopt the correct orientation for potent activation; and compounds **30, 31, 34, 38, 39, 40** and **50** do not apparently present the correct interacting functionality to the receptor as they are inactive. Indeed, of all the analogs designed to probe the SAR of region I, only compounds **29** and **33** are capable of activating FXR to a significant extent. Due to relative ease of synthesis of compound **29** this analog was chosen as a starting point for the modification of region II.

Evaluation of Benzopyran Region II SAR

[0069] As shown in Figure 10, the effect of numerous substitution patterns in this region of the molecule was examined (see Figure 11 for preparation of these compounds). Only compounds **65** (EC_{50} = 358 nM) and **68** (EC_{50} = . 1000 nM) were more effective than compound **29** in activating FXR. Substituted aromatic amide derivatives such as **69 – 77** were all found to be less active than the parent compound **68**. Alkyl derivatives **78** and **79** were inactive as were sulfonamide **82**, thiourea **84**, and thioamide **83**, suggesting the importance of acylation at this position. The sum of these results pointed to the desirable presence of moderately bulky cycloalkyl amide moieties in region II for good activity.

Evaluation of Benzopyran Region III SAR

[0070] Having thoroughly examined regions I and II, the modification of region III was then undertaken (see Figure 12 for structures and Figures 13 and 14 for preparation of compounds). Incorporation of polar H-bond donating functional groups such as those that adorn compounds **86, 93, 94, 98** and **100** did not improve the activity of the analogs. Nor did the addition of H-bond acceptors such as in **89, 90, 95, 99** and **101** improve the ability of the parent compound **68**

to activate FXR. Finally, the addition of bulky lipophilic groups to the benzopyran moiety afforded compounds that only weakly activated FXR. However, replacement of the double bond in the benzopyran unit by a dichlorocyclopropane unit provided analog **102** (EC_{50} = 333 nM). This potent compound was synthesized as depicted in Figure 15. Thus, benzopyran **103** was cyclopropanated under phase transfer conditions (adogen 464 (cat), NaOH, $CHCl_3$) and converted to the corresponding cinnamate via a Heck coupling ($Pd_2(dba)_3$, P(*o*-tol)$_3$, Et_3N) with methyl acrylate to yield **102**. Replacement of the benzoyl group in region II of compound **102** with the cyclohexylcarbonyl moiety afforded the even more potent compound **149** (EC_{50} = 188 nM).

[0071] Although compound **149** (EC_{50} = 188 nM) represents a significant improvement in potency over compound **65** (EC_{50} = 348 nM), it was not readily apparent how the activity of this class of compounds could be further improved. Therefore, it was decided to examine the effect of replacing the benzopyran moiety with other ring systems.

[0072] Figure 16 presents a series of compounds in which the benzopyran moiety was replaced with certain groups of varying molecular diversity (see Figures 17 and 18 for preparation). Biological assays showed that replacement of the benzopyran with a small aromatic unit generally had a detrimental effect on activity. For instance, compounds **110** and **112 – 117** (see Figure 19) were inactive, while compounds **111** and **118** showed only moderate activation of FXR (EC_{50} = 680 nM and 606 nM, respectively). However, replacement of the benzopyran with an aromatic ring bearing substituents at the para position produced compounds with improved activity. For example, 4-tert-butyl cinnamate **105** (EC_{50} = 127 nM), stilbenes **121** and **122** (EC_{50} = 36 and 208 nM, respectively), biaryls **124 – 127** (EC_{50} = 510, 69, 77, 227 nM, respectively) and aryl thiophenes **128** and **129** (EC_{50} = 206 and 256 nM, respectively) were all potent activators of FXR in the cell-based reporter assay. The synthesis of compound **105** is outlined in Figure 20 (see Figure 21 for the preparation of **121 – 129**). Thus, acylation of 3-bromoaniline ($C_6H_{11}COCl$, Et_3N) gave cyclohexylamide **131**. Subsequent reaction of **131** under Heck coupling conditions ($Pd_2(dba)_3$, P(*o*-tol)$_3$, Et_3N) with methyl acrylate gave **132**. Finally, alkylation (4-bromobenzyl bromide, NaH) of cinnamate **132** followed by a second Heck coupling ($Pd_2(dba)_3$, P(*o*-tol)$_3$, Et_3N) with tert-butyl acrylate gave **105**.

[0073] This initial survey of the three regions of SAR outlined in Figure 4 led to the identification of several potent FXR agonists for further evaluation. One such agonist is the benzopyran-derived dichlorocyclopropane **149** (EC_{50} = 188 nM). Compound **105** (EC_{50} = 127 nM) is an example of a bis-cinnamate derivative. Finally, compounds **121** (EC_{50} = 36 nM) and **124** (EC_{50} = 69 nM) are stilbene and biaryl derivatives, respectively, of invention compounds. Based on data presented in Figures 5, 10 and 12, compound **149** appeared to represent the most potent derivative that could be readily obtained among the benzopyran-derivatives. However, the bis-cinnamate, biaryl, and stilbene derivatives of invention compounds were thought to still possess considerable potential for further development and rigorous SAR analysis. Below the results of such investigations are detailed, which indeed led to further enhancement of biological activity.

Examination of the bis-cinnamate series

[0074] Similar to the results described above, the meta substituted methyl cinnamate moiety on the "right-hand" region of the molecule remained a desirable component for elevated activity among the bis-cinnamate derivatives of invention compounds (see Figures 19A and 22). Replacement of this methyl acrylate unit with either a methyl or ethyl allylic ether (compounds **136** and **137**) caused only a slight decrease in activity (EC_{50} = 243 and 220 nM, respectively). A marked decline in potency accompanied substitution of the methyl acrylate by more sterically bulky ethers or esters (compounds **133** and **134**) or amides (compound **135**). Interestingly, saturation of the acrylate olefin (compound **139**) afforded only a two-fold decrease in potency, EC_{50} = 274 nM, which supports the notion that conformational rigidity is a factor contributing to, but not essential for, high affinity ligands. Importantly, compound **139** suggests that the methyl acrylate moiety is not simply functioning as a latent electrophile.

[0075] Region II also closely mirrored the preceding data as cycloalkyl amides remained the preferred substituents (compounds **105** and **140** – **142**: EC_{50} = 127 – 250 nM) among the bis-cinnamate derivatives of invention compounds (see Figures 19B and 23). Aromatic and heterocyclic amides as well as alkyl ureas led to moderate potency (compounds **143** – **145**: EC_{50}

= 205 – 236 nM) whereas incorporation of bulky ureas such as compound **146** rendered compounds of only marginal efficacy.

[0076] As mentioned above, replacement of the benzopyran moiety with a benzyl group bearing a tert-butyl acrylate moiety in the para-position yielded compound **105** with dramatically increased efficacy (EC_{50} = 127 nM). Interestingly, placement of the same tert-butyl acrylate group in either the meta or ortho positions of the aromatic ring (compounds **107** and **109**, Figures 16 and 18 for synthesis) in Region III led to only micromolar potency. Further investigation of the "left-hand" region in this series of compounds demonstrated that a decrease in ester group size yielded a corresponding decrease in efficacy (EC_{50} of t-butyl > i-propyl > ethyl > methyl; compounds **105**, **150** – **152**, Figures 19D and 24). Similarly, substitution of the ester with either carboxylic acid or amide functionality provided less effective compounds with EC_{50} values in the micromolar range. Substitution of the tert-butyl acrylate moiety with a methyl or ethyl allylic ether (compounds **156** and **157**) retained considerable potency (EC_{50} = 233 and 198 nM, respectively). However, the more bulky phenyl allylic ether **158** possessed only micromolar activity. In addition, saturation of the acrylate moiety (compound **159**) showed a two-fold decrease in potency from the parent compound **105**. Finally, substitution of the ortho position of the aromatic ring of the tert-butyl acrylate series with oxygenated functionality (compounds **161** – **167**, see Figure 19D and Figure 25 for synthesis) afforded compounds with very low biological activity.

Construction of Biaryl and Stilbene Containing Focused Libraries

[0077] In an effort to further explore the activities of biaryl and stilbene derivatives of invention compounds, a 94-membered library of such compounds was constructed employing a solid phase strategy. As shown in Figures 26 and 27, Boc protected cinnamic acid **168** was immoblized on Merrifield resin (Cs_2CO_3) to afford resin **169**. The Boc group of this resin was removed by treatment with 20% TFA in CH_2Cl_2 and the resultant resin-bound amine was reductively alklylated with 4-bromobenzaldehyde ($NaCNBH_3$) to yield amino resin **170**. Resin **170** was acylated with one of three acyl groups to give amide or urea resins **171**. The acylated resins (**171**) were subjected to either Heck coupling ($Pd_2(dba)_3$, P(*o*-tol)$_3$, Et_3N) with thirteen substituted styrenes or Suzuki coupling ($Pd(PPh_3)_4$, Cs_2CO_3) with eighteen boronic acids to yield

stilbene resins **172** and biaryl resins **173**, respectively. The selection of appropriate styrenes and boronic acids for inputs into this combinatorial library was guided by initial comparisons of tert-butyl stilbene (compound **123**, EC_{50} = > 1000 nM) to the unsubstituted stilbene **102** (EC_{50} = 36 nM), and biaryl compound **124** (EC_{50} = 510 nM) to compound **125** (EC_{50} = 69 nM). It was reasoned that both the stilbene and the biaryl ligands needed to fit into the same region of space within the receptor site for potent activation. Thus, stilbenes in which the aromatic nucleus is removed two carbon atoms further away from the core of the molecule should be adorned with small substituents while the biaryl compounds should be adorned with larger functionality for optimal activity. Cleavage of resins **172** and **173** with NaOMe yielded methyl acrylates **121**, **125**, **126** and **174 - 263**. Analysis of the library by LCMS after purification showed the average purity of these compounds to be > 95%.

[0078] Screening of this compound library in the cell-based assay led to some intriguing results as summarized in Figure 28. Thus, it was found that in both stilbene and biaryl derivatives of invention compounds, analogs bearing the cyclohexylamide moiety are generally the most potent followed by those bearing the isopropyl amide or isopropyl urea units. As predicted above, stilbenes bearing smaller substituents were more potent than those bearing larger functionality. For instance, substituted stilbene **121** and mono-fluoro stilbenes **192**, **201**, and **204** were among the most active, while mono-methyl derivative **174** and tri-methyl derivative **195** were among the least active. Also of interest were heterocyclic compounds **207** and **210**, which retained good potency (EC_{50} = 309 and 227 nM, respectively) and may possess improved pharmacological properties. With biaryl derivatives of invention compounds, compounds which present more bulk at the terminus of the structure were more active. With these derivatives, compounds **259** (EC_{50} = 25 nM) and **244** (EC_{50} = 38 nM) were particularly active. Overall, most of the compounds synthesized in this follow-up library were efficient activators of FXR, confirming the accuracy of the working hypothesis for the FXR binding pocket described above, which provides a solid basis for further development of FXR activators.

[0079] A summary of the molecular requirements for potent FXR activation is shown in Figure 29. Thus, in region I the presence of the meta methyl acrylate unit is important for potent activation as only a few modifications retain good activity. The most potent compounds possess

a cycloalkylamide in region II. Finally, region III is the most tolerant and several structural elements were found to provide a good fit within the pocket of the receptor.

[0080] In order to determine how selectively the above-described compounds activated FXR, some of the most active compounds were screened against a panel of nuclear receptors. Most of these compounds were found to be selective for activation only of FXR. Notably, however, compound **149** also potently activated SXR. This result may lead to compounds which have utility in the treatment of diseases linked to the accumulation of toxic bile acids.

[0081] In accordance with another embodiment of the present invention, there are provided formulations comprising at least one of the above-described compounds in a pharmaceutically acceptable carrier therefor. Exemplary pharmaceutically acceptable carriers include solids, solutions, emulsions, dispersions, micelles, liposomes, and the like. Optionally, the pharmaceutically acceptable carrier employed herein further comprises an enteric coating.

[0082] Pharmaceutically acceptable carriers contemplated for use in the practice of the present invention are those which render invention compounds amenable to oral delivery, transdermal delivery, intravenous delivery, intramuscular delivery, topical delivery, nasal delivery, and the like.

[0083] Thus, formulations of the present invention can be used in the form of a solid, a solution, an emulsion, a dispersion, a micelle, a liposome, and the like, wherein the resulting formulation contains one or more of the compounds of the present invention, as an active ingredient, in admixture with an organic or inorganic carrier or excipient suitable for enterable or parenteral applications. The active ingredient may be compounded, for example, with the usual non-toxic, pharmaceutically acceptable carriers for tablets, pellets, capsules, suppositories, solutions, emulsions, suspensions and any other suitable for use. The carriers which can be used include glucose, lactose, gum acacia, gelatin, manitol, starch paste, magnesium trisilicate, talc, corn starch, keratin, colloidal silica, potato starch, urea, medium chain length triglycerides, dextrans, and other carriers suitable for use in manufacturing preparations, in solid, semisolid, or liquid form. In addition auxiliary, stabilizing, thickening, and coloring agents and perfumes may

be used. The active compound(s) is (are) included in the formulation in an amount sufficient to produce the desired effect upon the process or disease condition.

[0084] Invention formulations containing the active ingredient may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs. Formulations intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such formulations may contain one or more agents selected from the group consisting of a sweetening agent such as sucrose, lactose, or saccharin, flavoring agents such as peppermint, oil of wintergreen or cherry, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets containing the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients used may be, for example (1) inert diluents such as calcium carbonate, lactose, calcium phosphate or sodium phosphate; (2) granulating and disintegrating agents such corn starch, potato starch or alginic acid; (3) binding agents such as gum tragacanth, corn starch, gelatin or acacia, and (4) lubricating agents such as magnesium stearate, steric acid or talc. The tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed. They may also be coated by such techniques as those described in U.S. Pat Nos. 4,256,108; 4,160,452; and 4,265,874, to form osmotic therapeutic tablets for controlled release.

[0085] In some cases, formulations contemplated for oral use may be in the form of hard gelatin capsules wherein the active ingredient is mixed with inert solid diluent(s), for example, calcium carbonate, calcium phosphate or kaolin. They may also be in the form of soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example, peanut oil, liquid paraffin, or olive oil.

[0086] Invention formulations may be in the form of a sterile injectable suspension. This suspension may be formulated according to known methods using suitable dispersing or wetting

agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides, fatty acids, naturally occurring vegetable oils like sesame oil, coconut oil, peanut oil, cottonseed oil, etc. or synthetic fatty vehicles like ethyl oleate or the like. Buffers, preservatives, antioxidants, and the like can be incorporated as required.

[0087] Invention formulations may also be administered in the form of suppositories for rectal administration of the drug. These formulations may be prepared by mixing the drug with a suitable non-irritating excipient, such as cocoa butter, synthetic glyceride esters of polyethylene glycols, which are solid at ordinary temperatures, but liquefy and/or dissolve in the rectal cavity to release the drug. Since individual subjects may present a wide variation in severity of symptoms and each drug has its unique therapeutic characteristics, the precise mode of administration and dosage employed for each subject is left to the discretion of the practitioner.

[0088] Amounts effective for the particular therapeutic goal sought will, of course, depend on the severity of the condition being treated, and the weight and general state of the subject. Various general considerations taken into account in determining the "effective amount" are known to those of skill in the art and are described, e.g., in Gilman *et al.*, eds., Goodman And Gilman's: The Pharmacological Bases of Therapeutics, 8th ed., Pergamon Press, 1990; and Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Co., Easton, Pa., 1990, each of which is herein incorporated by reference.

[0089] The term "effective amount" as applied to invention compounds, means the quantity necessary to effect the desired therapeutic result, for example, a level effective to treat, cure, or alleviate the symptoms of a disease state for which the therapeutic compound is being administered, or to establish homeostasis. Since individual subjects may present a wide variation in severity of symptoms and each drug or active agent has its unique therapeutic characteristics, the precise mode of administration, dosage employed and treatment protocol for each subject is left to the discretion of the practitioner.

[0090] In accordance with yet another embodiment of the present invention, there are provided methods for modulating process(es) mediated by farnesoid X receptor polypeptides, said methods comprising conducting said process(es) in the presence of an effective amount of at least one compound according to the invention.

[0091] As employed herein, "modulating" refers to the ability of a modulator for a member of the nuclear receptor superfamily (*e.g.*, FXR) to either directly (by binding to the receptor as a ligand) or indirectly (as a precursor for a ligand or an inducer which promotes production of ligand from a precursor) induce expression of gene(s) maintained under hormone expression control, or to repress expression of gene(s) maintained under such control. Exemplary processes contemplated for modulation according to the invention include cholesterol metabolism, regulation of lipid homeostasis, stimulation of bile transport and absorption, regulation of the expression of genes involved in the excretion and transportation of bile acids (including intestinal bile acid-binding protein (IBABP)), bile salt export pump (BSEP) and canalicular multi-specific organic anion transporter (cMOAT), and the like.

[0092] Bile acids are derivatives of cholesterol synthesized in the hepatocyte. Cholesterol, ingested as part of the diet or derived from hepatic synthesis is converted into the bile acids cholic and chenodeoxycholic acids, which are then conjugated to an amino acid (glycine or taurine) to yield the conjugated form that is actively secreted into cannaliculi. Bile acids are facial amphipathic, that is, they contain both hydrophobic (lipid soluble) and polar (hydrophilic) faces. The cholesterol-derived portion of a bile acid has one face that is hydrophobic (that with methyl groups) and one that is hydrophilic (that with the hydroxyl groups); the amino acid conjugate is polar and hydrophilic. Therefore, compounds that can be used to modulate such pathways via effects on FXR involving bile acids are useful in cholesterol metabolism.

[0093] Bile acid synthesis is a major pathway for cholesterol disposal and thus represents a potential therapeutic target pathway for the treatment of hypercholesterolemia. FXR acts as a bile acid receptor and biological sensor for the regulation of bile acid biosynthesis. FXR is known to regulate cholesterol metabolism in two ways: (1) chenodeoxycholic acid (CDCA), a

primary bile acid, binds directly to and activates FXR, which then mediates the feedback suppression by bile acids of cholesterol 7 alpha-hydroxylase (CYP7A1), the rate-limiting enzyme in bile acid biosynthesis from cholesterol; and (2) FXR participates in the activation of intestinal bile acid binding protein (IBABP), which is involved in the enterohepatic circulation of bile acids. Thus FXR constitutes a potential therapeutic target that can be modulated to enhance the removal of cholesterol from the body. Novel compounds identified by the methods presented herein provide a new tool for regulating or modulating FXR function.

[0094] Furthermore, FXR is known to in turn activate a series of target genes. In particular FXR functions as a heterodimer with the 9-cis-retinoic acid receptor (RXR). A number of target DNA binding sequences that would be present in target genes have recently been identified. A consensus sequence has been determined, which contains an inverted repeat of the sequence AGGTCA with a 1-base pair spacing (IR-1) (Laffitte *et al.*, *J. Biol. Chem.* **275**:10638-10647 (2000). This sequence was shown to be a high affinity binding site for FXR/RXR *in vitro* and to confer ligand-dependent transcriptional activation by FXR/RXR to a heterologous promoter in response to a bile acid or synthetic retinoid. Although these studies demonstrated that the FXR/RXR heterodimer binds to the consensus IR-1 sequence with the highest affinity, it was also demonstrated that FXR/RXR can bind to and activate through a variety of elements including IR-1 elements with changes in the core half-site sequence, spacing nucleotide, and flanking nucleotides. In addition, it was shown that FXR/RXR can bind to and transactivate through direct repeats. Therefore, by providing novel ways to modulate FXR function, the present invention in turn provides a method of modulating the function of a variety of target genes that are acted upon by FXR.

[0095] In accordance with still another embodiment of the present invention, there are provided methods for the treatment of hypercholestemia, said methods comprising administering an effective amount of at least one compound according to the invention to a subject in need thereof.

[0096] In accordance with still another embodiment of the present invention, there are provided methods for the treatment of cholestasis, said methods comprising administering an

effective amount of at least one compound according to the invention to a subject in need thereof.

[0097] The invention will now be described in greater detail with reference to the following non-limiting examples.

EXAMPLE 1

In vivo assay

[0098] The feasibility of creating high throughput screens (HTS) for ORs was explored using FXR as a candidate orphan receptor (OR) with a known activator, chenodeoxycholic acid (CDCA) as a ligand. The screen is based on the co-transfection of a full-length receptor with the reporter vector containing a natural hormone response element under a minimal eukaryotic promoter. The results provided herein (see, for example, Figure 30) demonstrate that compounds can be successfully screened in a dose dependent manner for potential activating chemical ligands using a full length FXR on a natural response element. These results validate the robustness of the assay for FXR, in 384-well plates. Using this 384-well format, the high throughput screening (HTS) approach to FXR as a candidate OR was employed. For this test screen, a 10,000 membered library, constructed around the privileged 2,2-dimethylbenzopyran scaffold, was employed (see Nicolaou *et al.*, *J. Am. Chem. Soc.* **122**:9939-9953 and 9954-9967 (2000)). This library comprises approximately 10,000 distinct compounds with structures and sizes similar to natural products such as phyto-estrogens, flavanoids, coumarins and long chain fatty acids. A central question in the feasibility studies is whether this library is suitable for screening for nuclear receptor ligands. Samples of this library were first reformatted into a 384-well format and then subjected to the FXR cell-based assays described above and assessed for FXR-mediated transcriptional activity. Cells were exposed to approximately 10 μM of sample for 18 hrs prior to washing and luciferase analysis.

[0099] The 25 most active compounds at 10 μM were re-synthesized to confirm their structure and activity. Smaller "focused" chemical libraries were then designed and prepared around these hits and subjected to multiple rounds of screening. The design and rationale of

smaller and more focused libraries around the initial hits identified from primary screen is represented in Figure 4. Through this iterative process a total of seven additional rounds of synthesis and selection was conducted resulting in novel compounds that are as effective as a proprietary synthetic ligand developed by Glaxo-Smith-Kline (GW4064) in cell based assays. Using one of these identified compounds, fexaramate (EC_{50} 127 nM), as a scaffold, three additional focused libraries were made and screened to obtain at least four potent, non-steroidal FXR agonists termed fexarene (EC_{50}, 36 nM), fexaramine EC_{50}, 36 nM), fexarine (EC_{50}, 25 nM) and fexarchloramide (EC_{50}, 188 nM). EC_{50} values were determined with Prism 3.0 software via the activity of the subject compound in the previously described cell based assay.

EXAMPLE 2

In vitro Screening

[0100] An *in vitro* based "proximity" screen is an excellent complement to live cell assays and can be used as a measure of direct ligand binding. Hence this type of screen is also an effective measure of the affinity of binding without the use of a radiolabel. The approach employed herein is termed AlphaScreen technology. For this assay purified receptor protein is expressed as a glutathione S-transferase (GST) fusion protein and is bound via a GST antibody to a "donor" bead. This is then mixed with a biotinylated co-activator peptide that has been linked to an Avidin proximity sensitive "acceptor" bead. These reactants are mixed in a 384-well plate and are then exposed to either a known inducer (control) or an ordered array of unknown compounds (library). If the acceptor bead (linked to the co-activator peptide) is brought into close proximity of the donor bead, by virtue of a biological interaction, singlet-state oxygen molecules are released and react with chemiluminescent groups in the acceptor beads. The effect of either known inducers or candidate chemical compounds on the interaction of a receptor and its co-activator peptide can be measured by a change in the Alpha signal.

[0101] The ability of the *in vitro* AlphaQuest assay to detect receptor/co-activator peptide interactions in a 384 well format has been evaluated using the thyroid hormone receptor (TR) and the retinoid X receptor (RXR) as positive controls. The results demonstrate that receptor/co-activator peptide interactions can be detected in a dose-dependent manner with binding

efficiencies similar to those reported in the literature, validating this as a critical *in vitro* approach to demonstrate binding of candidate ligands in the absence of a high affinity radiolabeled competitor.

[0102] It will be apparent to those skilled in the art that various changes may be made in the invention without departing from the spirit and scope thereof, and therefore, the invention encompasses embodiments in addition to those specifically disclosed in the specification, but only as indicated in the appended claims.